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IMMUNOPHARMACOLOGY AND IMMUNOTOXICOLOGY, 13(4), 531-550 (1991)

MODIFICATION OF MITOGEN-INDUCED PROLIFERATION OF MURINE SPLENIC LYMPHOCYTES BY *in vitro* TOCOPHEROL

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ABSTRACT

The effect of α-tocopherol on *in vitro* proliferation of murine splenic lymphocyte cultures supplemented with various concentrations of the vitamin has been measured at sub-optimal, optimal and supra-optimal levels of the T-cell mitogen Concanavalin A (Con A). In the concentration range (1-25 μg/ml), tocopherol enhanced proliferation when administered up to 24 hours after exposure to sub-optimal and optimal concentrations of Con A; however, at supra-optimal levels of the mitogen, it appeared to inhibit proliferation. In the concentration range 50-100 μg/ml, tocopherol supplementation only enhanced proliferation in response to sub-optimal concentration of Con A. The spontaneous proliferation of lymphocytes in the absence of mitogens was increased by tocopherol supplementation at all concentrations tested. In contrast, there appeared to be only slight stimulation of B-cell proliferation in response to optimal concentration of bacterial lipopolysaccharide (LPS) by lower levels of vitamin E. Tocopherol supplementation of cultures over a broad range of concentrations (0.5 - 100 μg/ml) had no significant effect on cell viability before onset of proliferation at 18 hours after exposure to Con A, nor was there evidence of earlier onset of DNA synthesis in response to mitogen in the presence of 5 μg/ml of the

[1]List of Abbreviations: ANOVA, Analysis of Variance; Con A, Concanavalin A; FCS, Fetal Calf Serum; LPS, Lipopolysaccharide; 2-ME, 2-Mercaptoethanol; TdR, Thymidine; PHA, Phytohemagglutinin; PMA, Phorbol myristic acetate.

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vitamin. Although macrophage depletion of cultures impaired proliferation induced by Con A, tocopherol supplementation continued to stimulate proliferation at optimal and sub-optimal levels of mitogen.

INTRODUCTION

There are numerous reports concerning the modification of vertebrate immune responses by various forms of vitamin E both in vivo by dietary restriction and supplementation, as well as by injection of pharmacological doses of tocopherol (1-5). Adequate vitamin E is required for normal expression of both humoral and cell-mediated responses (4,5). These functions are depressed in tocopherol-deficient animals and are enhanced by pharmacological doses of vitamin E (6-10).

Severe depression of blastogenic responses to both B- and T-cell mitogens has been observed in lymphocytes derived from animals maintained on vitamin E deficient diets (1-5,11). Exogenous tocopherol added directly to lymphocyte cultures enhanced proliferation. Other studies have demonstrated that proliferative responses in lymphocytes stimulated with polyclonal mitogens can be potentiated by tocopherol (12). On the other hand, there is some evidence that high concentrations of tocopherol inhibited response to phytohemagglutinin (PHA)' and suppressed mixed lymphocyte reaction to allogenic antigen (13,14). Corwin et al. (15-17) reported that dietary supplementation and in vitro augmentation of tocopherol levels stimulated proliferative response to sub-optimal Con A', but response to optimal concentration of mitogen was unaffected.

In the absence of mitogens, tocopherol (1 - 5 μM) was observed to be slightly mitogenic to a degree comparable to that observed with mercaptoethanol (2-ME')(15-17). Murine splenic lymphocytes depleted of adherent cells (macrophages and monocytes) demonstrated impaired proliferative response to mitogens (16); however, the response could be restored to a considerable degree by in vitro supplementation with tocopherol (1 μg/ml).

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The mechanism(s) by which lymphoproliferative activity is stimulated by tocopherol remains obscure. Non-specific general antioxidant-based effects (18-20) may be involved since other antioxidants have slight mitogenic effects and can enhance response to mitogens (15,17). Regulation of prostaglandin biosynthesis (a specific antioxidant function of tocopherol) may also play a significant role (12,15-17). Tocopherol supplementation suppresses prostaglandin synthesis, particularly prostaglandin E series, important inhibitors of antibody response and T-cell blastogenesis (21,22). On the other hand, Corwin et al. (15) did not observe inhibition of prostaglandin biosynthesis in mitogen-stimulated murine spleen cell cultures derived from tocopherol-supplemented animals or in cultures supplemented with vitamin E in vitro. Likewise, indomethacin, a known inhibitor of the arachidonic acid peroxidation step of prostaglandin synthesis, did not potentiate responsiveness to sub-optimal Con A.

The well-established role of tocopherol in maintaining membrane structural and functional integrity may have some impact on the number and location of binding sites for antigens and interleukins on the surface of lymphocytes which might account for the modifying effects of tocopherol on immune functions. The study to be described presents additional observations on the response of lymphocytes to tocopherol which may help to elucidate the immunomodulating effects of tocopherol.

MATERIALS AND METHODS

Isolation of Splenic Lymphocytes

C57/Bl female mice (16-20 g) were killed by CO_2 inhalation and immediately immersed in 70% ethanol. Spleens were removed under sterile conditions and placed in 10 ml of cold RPMI-1640 tissue culture medium containing L-glutamine, 25 mM Hepes buffer and 2% penicillin-streptomycin solution containing 5000 IU/ml penicillin and 5000 μg/ml streptomycin (Gibco). Spleens were teased free of fat and connective tissue and washed several times by serial passage through culture medium in sterile culture plates. Spleens were

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gently scraped with a scalpel to release a cell suspension. The suspension was passed through a fine nylon mesh and allowed to settle for 15 minutes. The supernatant was centrifuged at 200 X g for 10 minutes. Red blood cells were removed by lysis in Tris ammonium chloride pH 7.2 (23) which was added to pellets (10 ml per ml of pellet). After resuspension at room temperature, a layer of 2.0 ml of fetal calf serum (FCS') was introduced under the suspension. This was centrifuged for 10 minutes at 300 X g. The spleen cell pellet was resuspended and washed in 10 ml of serum-free medium by centrifugation and finally resuspended in 2-5 ml of medium.

Viable lymphocyte concentration was determined by mixing 100 µl of cell suspension with an equal volume of 0.2% trypan blue in 0.85% NaCl. Cells were counted using a Neubauer hemacytometer and at least 250 cells were scored. Lymphocyte cultures were established using spleen suspensions containing greater than 95% viable cells. Cell suspensions were diluted to 1 X 10^7 cells/ml.

Culture of Splenic Lymphocytes

Cells were cultured in RPMI-1640 medium as described above, supplemented with 10% FCS. Lymphocytes were cultured in 2.0-ml cultures in 16 X 125 mm polystyrene tissue-culture tubes (Corning, N.Y.) and in 200-µl cultures in 96-well flat-bottom microwell culture plates (Linbro, Flow). Approximately 2.0 - 2.5 X 10^6 cells/ml were used in all cases. Tube-cultures were individually flushed with 5% CO_2, tightly capped and held at a 45° angle at 37°C. Microwell cultures were maintained under 5% CO_2, 100% RH at 37°C. Lyophilized sterile Concanavalin A type IV-S (Sigma) and lyophilized lipopolysaccharide (LPS') derived from S. typhosa (Difco) were reconstituted in RPMI-1640 (1 mg/ml). Cultures were supplemented with various concentrations of Con A (0.5 to 10 µg/ml) and LPS (1-25 µg/ml) immediately or at specified times before addition of tocopherol or other test-compounds. DL-α-tocopherol (ICN) was solubilized in FCS as described by Narayanareddy and Murthy (24).

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Stock solutions (1 mg/ml) were added to cultures containing 10% FCS to achieve concentrations from 0.5 - 100 μg/ml.

Incorporation of ^{3}H-Thymidine

Thymidine [methyl-^{3}HTdR], specific activity 20-40 Ci/mmole (ICN or NEN), was added to cultures at 48 hours following exposure to mitogen. In the case of the 2.0-ml tube-cultures, 2.0 μCi of label was added, and in the case of 200-μl cultures, 0.1 μCi was added. After 18 hours, cultures were removed from the incubator, chilled and harvested by precipitation in 10% cold trichloroacetic acid, then collected on Whatman GF/A glass microfiber filter disks (tube-cultures) and by high-speed centrifugation (microwell-cultures). Precipitates on filters or pellets in microcentrifuge tubes were washed with ethanol and solubilized in Protosol (NEN). Samples were counted in Aquasol (NEN) or Cytoscint (ICN) using an LKB Rackbeta model 1517 liquid scintillation spectrometer. Absolute activity was determined using channels-ratio quench correction method.

RESULTS

The incorporation of thymidine during an 18-hour feeding at 48-66 hours following addition of Con A to splenic lymphocyte cultures has been measured as a function of both concentration of mitogen and of tocopherol added immediately after Con A. The magnitude of stimulation of proliferation by the mitogen varied from the spontaneous level (0 μg/ml) to an optimal response of 10-50 times this level. The optimal concentration observed in this study appears to be approximately 2.0 - 2.5 μg/ml. The data presented in Figure 1 summarize these responses and are expressed as relative incorporation for each treatment combination of mitogen and tocopherol, as a percent of incorporation in the cultures with 2.0 μg/ml Con A without tocopherol supplementation.

Supplementation of cultures with tocopherol at sub-optimal Con A concentrations resulted in significant ($P<0.1$; t-test) stimulation of proliferation at both physiological (5 μg/ml) and

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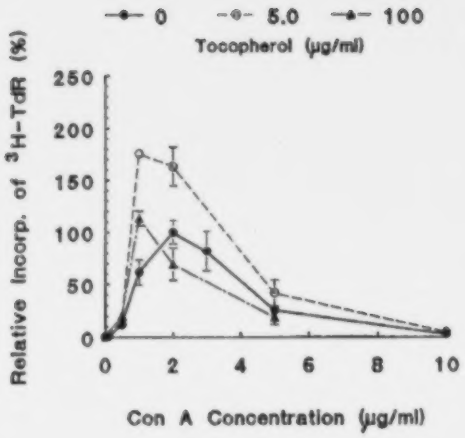

FIG. 1 Incorporation of ^{3}H-thymidine into lymphocytes as a function of Con A concentration measured in cultures supplemented with 0, 5 and 100 µg/ml tocopherol. Means and standard errors are presented. Incorporation is expressed as a percentage of uptake in cultures with 2.0 µg/ml Con A and 0 µg/ml tocopherol. Data has been derived from 3-5 separate measurements using triplicate cultures for each combination of Con A and tocopherol.

pharmacological concentrations (100 µg/ml). The situation is somewhat different at optimal Con A concentration (2 µg/ml); 5 µg/ml tocopherol stimulated blasting response, whereas higher concentrations (100 µg/ml) tended to inhibit this response. However, at 5-10 µg/ml Con A, tocopherol supplementation did not significantly modify responses ($P>.05$, t-test).

In Fig. 2, data are presented concerning proliferation relative to the non-tocopherol control group for various concentrations of tocopherol given immediately after 2.0 µg/ml Con A (optimal concentration of mitogen). Significant stimulation of proliferation by tocopherol treatments from 5 to 25 µg/ml was observed and significant inhibition was seen at both 50 and 100 µg/ml ($P<0.01$, ANOVA[1] and t-test).

It was interesting to note (Fig. 3) that spontaneous proliferation in the absence of mitogen was significantly stimulated

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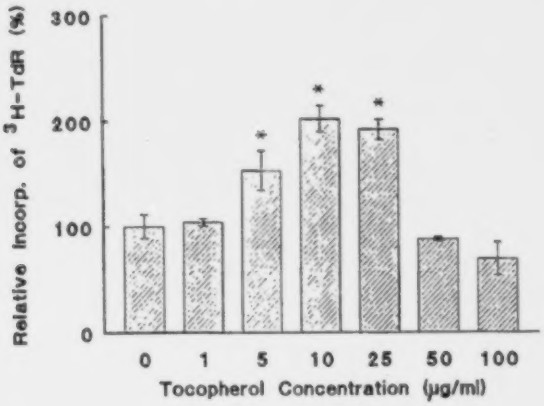

FIG. 2 Incorporation of ^{3}H-thymidine into lymphocytes in response to 2.0 µg/ml Con A as a function of tocopherol concentration in culture media. Incorporation is expressed as a percentage of the 0 µg/ml tocopherol group. Means and standard errors are presented for each concentration of tocopherol derived from data from 3-5 separate experiments using triplicate cultures. Significant stimulation occurred from 5 to 25 µg/ml tocopherol (*).

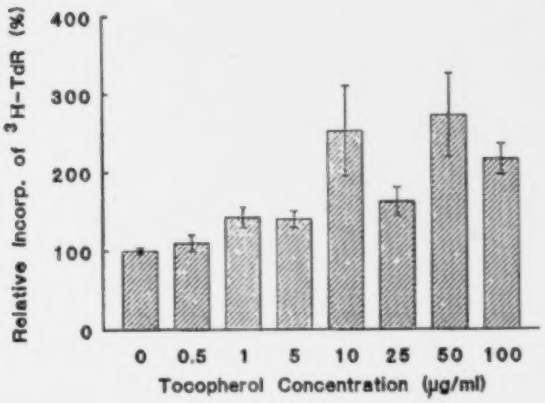

FIG. 3 Incorporation of ^{3}H-thymidine into lymphocytes in cultures not treated with mitogen but supplemented with various concentrations of tocopherol. Incorporation is expressed as a percentage of 0 µg/ml tocopherol group. Means and standard errors have been computed for each concentration derived from data obtained in 3-5 separate experiments using triplicate cultures.

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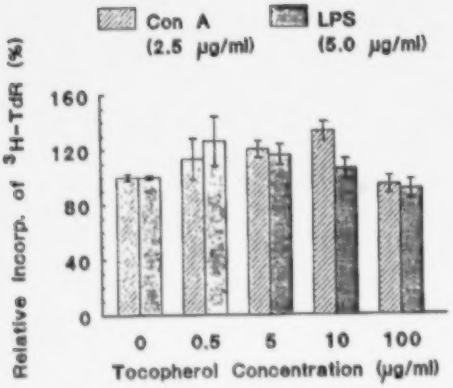

FIG. 4 Incorporation of ^{3}H-thymidine into lymphocytes in response to optimal concentrations of mitogen (2.5 μg/ml Con A or 5 μg/ml LPS) cultured with various concentrations of tocopherol expressed as a percentage of uptake by 0 μg/ml group. Means and standard errors are presented for data derived from 2-6 separate experiments using triplicate cultures.

($P<.01$; ANOVA and t-test) by tocopherol concentrations, even as low as 1 μg/ml, and remained elevated even at the highest concentration tested (100 μg/ml). The incorporation of ^{3}H-thymidine into cultures containing various concentrations of tocopherol but no mitogen are presented as percent incorporation in the absence of tocopherol.

In contrast to spontaneous and Con A-induced proliferation, which were affected by changes in tocopherol concentration of culture medium, response to the B-cell mitogen LPS in the lower range of its optimal concentration was only slightly modified by variations in tocopherol concentration (Fig. 4). The incorporation of ^{3}H-thymidine in response to 5 μg/ml LPS is presented for each concentration of tocopherol as a percentage of the untreated (0 μg/ml) control group. Small stimulatory effects on LPS-induced proliferation at 5 and 10 μg/ml are not statistically significant (ANOVA) nor is the apparent inhibitory effect at 100 μg/ml.

The effect of tocopherol supplementation on the time between Con A presentation and commencement of cell division has been

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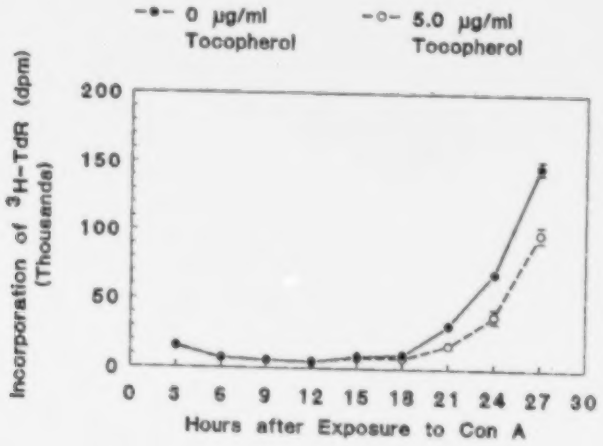

FIG. 5 Incorporation of ^{3}H-thymidine (3-h feedings) at various times following exposure to Con A (dpm). Mean and standard errors are presented for a single experiment with triplicate cultures at each time.

investigated and it was found that DNA synthesis began after 18 hours following treatment with the mitogen (Fig. 5). There was no evidence of earlier onset of proliferation with tocopherol treatment. In early stages of proliferation, the tocopherol-supplemented cultures exhibited lower levels of DNA synthesis. This is, however, based on a single experimental series with triplicate measurements at each time sampled.

Cell viability measured by trypan blue dye-exclusion has been assessed at 18 hours after Con A exposure in the presence of various tocopherol concentrations. Data have been pooled from several experiments with a minimum of 500 cells scored at each concentration. Viability was approximately 70% in untreated control cultures and no significant change in viability was observed prior to onset of cell division that might account for the effects of tocopherol on proliferation (Chi-square 2X2 contingency tests). The inhibitory effects of adding high concentrations of tocopherol on proliferation are not reflections of apparent cytotoxic effects prior to onset of division.

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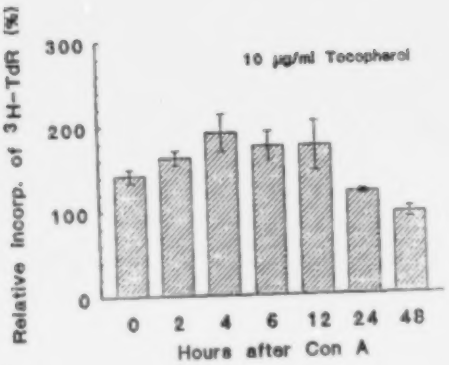

FIG. 6 Incorporation of ^{3}H-thymidine into lymphocytes treated with 10 µg/ml tocopherol at various times following addition of Con A expressed as a percentage of uptake by cultures without tocopherol. Mean and standard errors are indicated for data derived from 3-5 separate experiments using quadruplicate cultures at each time studied.

It was of interest to determine the time-course of effectiveness of tocopherol on proliferative response to Con A. The addition of tocopherol was delayed for various times up to 48 hours (immediately preceding addition of ^{3}H-thymidine). As indicated in Fig. 6, proliferation relative to the non-tocopherol control was significantly elevated ($P<.01$; ANOVA and t-test) at all times from 0 - 24 hours (although somewhat reduced in magnitude at 24 h); however, it was not stimulated when tocopherol was added 48 hours after mitogen. Thus, tocopherol present during the 18-h incorporation of labelled thymidine had no impact on DNA synthesis.

Other antioxidants also potentially stimulate immune functions. Some comparisons have been made with 2-mercaptoethanol (2-ME) which has long been known to stimulate proliferation of lymphocyte cultures (23). In Fig. 7, it is apparent that 2-ME had a large stimulatory effect on proliferation ($P<.01$; ANOVA); incorporation is plotted as natural Log value (ln dpm). The effect

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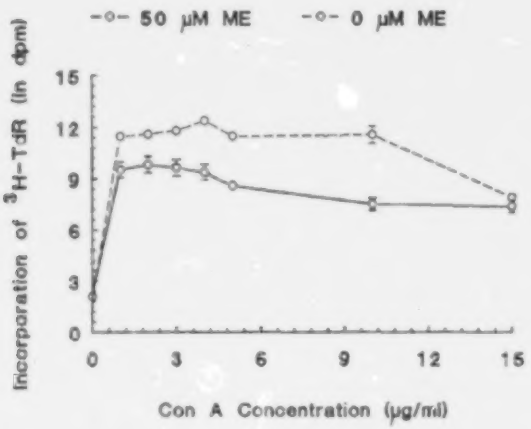

FIG. 7 Incorporation of ^{3}H-thymidine, plotted as the natural log (ln dpm), at various concentrations of Con A in the presence (5 μM) and absence of 2-mercaptoethanol. Means and standard errors are indicated for data derived from 3 separate experiments with 2-4 replicates at each concentration of Con A.

was prominent both above (3-10 μg/ml) and below (1 μg/ml) the optimum mitogen concentration. In this latter respect, there is a marked difference with the response to tocopherol.

The dependency on macrophage and other adherent accessory cells for the modifying effects of tocopherol was investigated in one experimental series (triplicate cultures) in which the responses to 0.5 μg/ml and 2.0 μg/ml Con A were compared in cultures with reduced numbers of adherent cells with and without tocopherol supplementation (5.0 μg/ml). A large reduction in adherent cell number (>90%) impaired proliferative response to both sub-optimal and optimal Con A (30% and 78% of normal levels, respectively). Tocopherol supplementation (5 μg/ml) increased proliferative responses for both concentrations of Con A (0.5 and 2.0 μg/ml) to 45% and 90% of normal levels, resp..

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DISCUSSION

Modification of Con A Response by Tocopherol

The polyclonal mitogen Con A binds to the surface receptors of T and B lymphocytes and accessory cells (25,26). Con A stimulates primarily proliferation of T-cells; however, some indirect stimulation of B-cell proliferation may occur at later stages of culture (50-60 h). Tritiated thymidine uptake in cultures containing both T and B-cells is reported to increase significantly 16-18 hours after presentation of the mitogen and attains maximal activity at approximately 48 hours. This was confirmed in the present study. In view of the fact that cultures were normally fed ^{3}H-thymidine from 48-66 h, the proliferation can be safely assumed to be almost exclusively confined to T-cell activity.

It has been demonstrated that murine spleen cells cultured with 5-10% fetal calf serum exhibit a broad dose-response profile to Con A. Anderson et al. (25) reported significant mitogenic stimulation in the concentration range 1-10 μg/ml with maximum proliferation at 5 μg/ml. In other work with C57Bl mice (27), spleen cells responded to 0.5-6 μg/ml and the optimum was found at 2 μg/ml, in excellent agreement with data presented in Fig. 1.

Consistent with the observations of Corwin et al (15-17), mitogenic responses produced by sub-optimal (0.5 μg/ml) Con A were significantly enhanced by supplementing with tocopherol at all concentrations tested (1-100 μg/ml). On the other hand, at optimal Con A (2-2.5 μg/ml), smaller but statistically significant stimulation by 1-25 μg/ml tocopherol was observed (Fig. 2). The mitogenic response generated by supra-optimal Con A was also enhanced to some extent by 5 μg/ml tocopherol. These findings represent a departure from existing reports in the literature indicating that the stimulatory effects of tocopherol on Con A mitogenesis are selective and limited to sub-optimal Con A. The stimulatory effect of tocopherol on Con A over the range 1-5 μg/ml is in marked contrast to the stimulatory effect of 2-ME which confers large increases in proliferation above and below the Con A

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optimum (Fig. 7). The mechanism of action of mercaptoethanol in stimulating proliferation probably differs from that of tocopherol.

It is interesting to note that stimulation of Con A-induced proliferation can be produced by supplementing cultures with relatively low levels of tocopherol, which coincide with the normal physiological range in mouse serum. It has been reported that plasma tocopherol levels in mice maintained on commercial diets containing adequate levels of vitamin E range from 5 to 12 μg/ml. Furthermore, tocopherol content of fetal calf serum has been reported to be less than 1 μg/ml (15-17); therefore, in cultures with 10% FCS, 0.1 μg/ml could be present. The optimal response to tocopherol seems to be 10-25 μg/ml and is consistent with the report of Bendich et al. (10) demonstrating that tocopherol levels greater than those required to prevent symptoms associated with classical vitamin E deficiency in rats (4-7 μg/ml) are needed for optimal response to mitogens.

Mitogenic Effects of Tocopherol

The modification of Con A mitogenesis in murine spleen cells by tocopherol in vitro has been previously reported (15-17). Physiological concentrations (e.g., 1 μg/ml) enhanced responses to 0.6 μg/ml Con A but had no significant stimulatory effect at optimal concentration. The authors further reported that tocopherol was slightly mitogenic in the absence of Con A to a similar degree as 2-mercaptoethanol. As indicated in Fig. 3, tocopherol in vitro was slightly mitogenic in the absence of mitogen; this does not seem to be the case with 2-ME (Fig. 7). Statistically significant mitogenic response was obtained in both the physiological (1-25 μg/ml) and the pharmacological range (50-100 μg/ml) for tocopherol. The magnitude of the effect on spontaneous DNA synthesis was much smaller than the increase caused by tocopherol on Con A-induced proliferation. In fact, there are some apparent differences; most notably, the inhibitory effects of 50-100 μg/ml at optimal Con A were not seen in

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the case of spontaneous proliferation. This is consistent with the observation that the inhibitory effects of high concentrations of tocopherol are also not seen at sub-optimal Con A (Fig. 2).

Effect of Tocopherol on B-cell Proliferation

In contrast to the significant stimulatory effects on spontaneous and Con A-induced proliferation, there is relatively small stimulation of B-cell proliferation in response to the B-cell mitogen LPS (Fig. 4) in the presence of various concentrations of vitamin E. This may suggest that B-cells are not likely contributing to the mitogenic effect of tocopherol. No attempt was made to investigate the effect on sub-optimal LPS situations. Others have reported stimulation of B-cell proliferation by tocopherol (1,3); however, it is generally observed that T-cells are more sensitive to vitamin E, which is consistent with our observations.

Effect of Vitamin E on Blasting Time-Course

A number of explanations may be proposed to account for the stimulatory effect of tocopherol on proliferation measured at 48-66 h after initiation of cultures. An earlier onset of cell division in tocopherol-supplemented cultures might effectively increase the number of cells proliferating during the thymidine feeding. This possibility seems to be ruled out by time-course data presented in Fig. 5 which indicate that the tocopherol-supplemented group did not begin to divide sooner.

Effect of Tocopherol on Lymphocyte Viability

Another possible explanation concerns a potential protective effect of vitamin E on lymphocyte viability (reduction in programmed cell death) in culture prior to the onset of division. When

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cultures were established, cell viability was greater than 95% and at 18 hours (just prior to the start of replicative DNA synthesis) viability was approximately 70%. This is due to both programmed cell death as well as possible toxic effects of atmospheric oxygen and oxidizing free radicals. Viability was not significantly altered by addition of various concentrations of tocopherol even though these resulted in both stimulatory and inhibitory effects on proliferation. Changes in viability do not seem to account for modifying effects on proliferative responses.

Time-Course of Responsiveness to Tocopherol

The mechanism(s) of tocopherol stimulation of Con A blastogenesis is not known. At one level, it seems that tocopherol effects are not involved in the early stages of mitogen-binding and secondary messenger effects associated with the first 6-8 hours after binding Con A. Tocopherol supplementation up to 12 hours after Con A exposure conferred full stimulatory effects; these effects were slightly reduced by 24 hours and absent at 48 hours. Clearly tocopherol *per se* in culture medium during ^{3}H-thymidine uptake (48-66 h post-mitogen) did not stimulate incorporation.

Role of Adherent Cells

It is known that macrophages play an essential role in the mitogenic effect of Con A on lymphocytes. If the action of tocopherol was on macrophage function, one might expect that cultures with substantially reduced numbers of macrophages would be less responsive to mitogen and to modification by tocopherol. Although the former was observed (reduced blastogenic response), the latter was not. In fact the opposite seemed to be the case, with macrophage-depleted cultures exhibiting somewhat greater response to tocopherol, particularly at sub-optimal Con A. Similar results have been reported by Corwin *et al*. (16).

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Similarity to Effects by 2-Mercaptoethanol

Tocopherol shares a functional resemblance to 2-mercaptoethanol (28) and phorbol myristic acetate (PMA)(29,32) in its ability to replace interleukin 1 and promote synthesis of interleukin 2 by helper T-cells; these effectively support lymphoproliferative responses to Con A in the relative absence of accessory cells. There are, however, two notable differences between the effects of vitamin E and 2-ME on spontaneous and Con A-induced proliferation. Mercaptoethanol maintains near-optimal response over a broad range of Con A concentrations; tocopherol tends to amplify the inhibitory effects of supra-optimal levels of the mitogen. But while tocopherol appeared to be mitogenic in the absence of Con A, there was no significant stimulation of spontaneous proliferation by 2-ME alone.

Inhibitory Effects of High Concentration of Tocopherol

There are studies reporting inhibition of mitogenesis in murine spleen cells by tocopherol. Yasunaga *et al*. (30) demonstrated that mitogenic responses produced by Con A, PHA and LPS were significantly suppressed in C3H/He mice injected *ip* with 80 IU/kg tocopherol daily for 14 days yielding serum tocopherol levels greater than 20 μg/ml.

At least one explanation can be proposed to account for the suppression of Con A mitogenesis produced by pharmacological doses of tocopherol *in vitro*. Higher concentrations of Con A are cytotoxic and this effect may be potentiated at higher tocopherol levels. There seems to be some evidence of synergistic interaction between tocopherol and Con A (e.g., shifting of optimum to a lower concentration of Con A). The magnitude of tocopherol stimulation was greatest below the Con A optimum and became progressively less with higher Con A. Secondly, it is also possible that tocopherol by itself is cytotoxic *in vitro* at pharmacological concentrations and in this respect might inhibit mitogenic responses independently of

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Con A. It has been demonstrated that tocopherol (50-100 μg/ml) was cytotoxic to human peripheral blood lymphocytes (24), increasing progressively over 96 hours; rate of cell death was maximal at 48-78 hours. In the present study, viability was checked just prior to onset of cell division and there was no evidence of increased or decreased survival. This does not preclude toxic effects being manifested at later times; however, interpretation of viability data is far more complex after onset of proliferation; this adds newly-formed cells to the population which affects average cell age.

Mechanisms of Vitamin E Effects on Lymphocytes

Several hypotheses can be advanced to explain the stimulation of Con A mitogenesis by tocopherol in relation to its antioxidant properties. It may protect membrane lipids from peroxidation by oxidizing radicals and active oxygen species (18). Another possible antioxidant mechanism may involve regulation of prostaglandin biosynthesis (15,17,21,31). In this regard, it has been reported that vitamin E inhibits synthesis of prostaglandin E2 which is known to suppress immune functions (22). Another suggestion proposes that tocopherol, like insulin, may stimulate mitogenic responses to Con A by maintaining (protecting) interleukin 2 receptors on the surface of lymphocytes (32). It has been suggested that tocopherol increases sensitivity of T-lymphocytes to Con A; this should result in a shift in the concentration-response profile, and there is some indication of this in the data. The Con A optimum appears to be shifted to lower concentrations at both 5 and 100 μg/ml tocopherol.

ACKNOWLEDGEMENT

This work was supported by research grants from the Natural Sciences and Engineering Research Council (Canada) and the Department of National Defence (Canada). The technical assistance of D. Little, J. Attwood, P. Cuscuna and P. Kauler is gratefully acknowledged.

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REFERENCES

1. Bendich, A., Antioxidant vitamins and immune responses, in: Contemporary Issues in Clinical Nutrition, II. Nutrition and Immunology, ed. R.K. Chandra, p. 125, Allan R. Liss Inc., New York, 1988.

2. Panush, R.S. and Delafuente, J.C., Vitamins and immunocompetence, in World Review of Nutrition and Dietetics, ed. G.H. Bourne, vol. 45, p. 97, Karger, New York, 1985.

3. Bendich, A., Antioxidant vitamins and their functions in immune responses, in: Antioxidant Nutrients and Immune Functions, eds. by A. Bendich, M. Philips and R.P. Tengerdy, p. 36, Plenum Press, New York, 1990.

4. Tengerdy, R. P., Immunity and disease resistance in farm animals fed vitamin E supplement, in: Antioxidant Nutrients and Immune Functions, eds. A. Bendich, M. Phillips and R.P. Tengerdy, p. 103-110, Plenum Press, N.Y., 1990.

5. Tengerdy, R.P., The role of vitamin E in immune response and disease resistance, in: Micronutrients and Immune Function, eds. A. Bendich, R.K. Chandra and G.T. Keusch, N.Y. Acad. Sci. 587: 25, 1990.

6. Marsh, J.A., Dietert, R.R. and Combs, G.F. Jr., Influence of dietary selenium and vitamin E on the humoral immune response of the chick, Proc. Soc. Exp. Biol. Med. 166: 228, 1981.

7. Gebremichael, A., Levy, E.M. and Corwin, L.M., Adherent cell requirement for the effect of vitamin E on in vitro antibody synthesis, J. Nutr. 114: 1297, 1984.

8. Saxena, Q. B., Saxena, R. K. and Adler, W. H., Effect of feeding a diet with half the recommended levels of all vitamins on the natural and inducible levels of cytotoxic activity in mouse spleen cells, Immunology 52: 41, 1984.

9. Bendich, A., Gabriel, E. and Machlin, L.J., Depression of rat and guinea pig lymphocyte blastogenic response by vitamin E deficiency: A new model for reproducible immune modulation, Ann. N. Y. Acad. Sci. 435: 383, 1984.

10. Bendich, A., E. Gabriel, E. and L.J. Machlin, L.J., Dietary vitamin E requirement for optimum immune responses in the rat, J. Nutr. 116: 675, 1986.

11. Tengerdy, R. P., Effects of vitamin E on immune responses, in: Vitamin E: A Comprehensive Treatise, ed. J. Machlin, p. 429, M. Dekker, New York, 1980.

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12. Sheffy, B.E. and Schultz, R.D., Influence of selenium and vitamin E on immune response mechanisms, Fed. Proc. 28: 2139, 1979.

13. Mann, P.L. and J.W. Logan, J.W., Suppression of the mixed lymphocyte reaction by alpha-tocopherol, N.Z. Med. J. 72: 31, 1970.

14. Prasad, J.S., Effect of vitamin E supplementation on leukocyte function, Amer. J. Clin. Nutr. 33: 606, 1980.

15. Corwin, L.W. and Shloss, J., Role of antioxidants on the stimulation of the mitogenic response, J. Nutr. 110: 2497, 1980.

16. Corwin, L.W., Gordon, R. K. and Shloss, J., Studies on the mode of action of vitamin E in stimulating T-cell mitogenesis, Scand. J. Immunol. 14: 565, 1981.

17. Corwin, L.M. and Shloss, J., Influence of vitamin E on the mitogenic response of murine lymphoid cells, J. Nutr. 110: 916, 1980.

18. Tappel, A.L., Vitamin E and free radical peroxidation of lipids, Ann. N.Y. Acad. Sci. 203: 12, 1972.

19. Green, J., Vitamin E and the biological antioxidant theory, Ann. N.Y. Acad. Sci. 203: 29, 1972.

20. McKay, P. B. and King, M. M., Vitamin E: its role as a biologic free radical scavenger and its relationship to the microsomal mixed-function oxidase system, in: Vitamin E: A Comprehensive Treatise, ed. L.J. Machlin, p. 289, Marcel Dekker, New York, 1980.

21. Goodwin, J.S. and Webb, D.R., Regulation of the immune response by prostaglandins, Clin. Immunol. Immunopath. 15: 106, 1980.

22. Meydani, S., Meydani, N., Verdon, M., Shapiro, C.P., Blumberg, A. A. and Hayes, K. C., Vitamin E supplementation suppresses prostaglandin E synthesis and enhances immune system of aged mice. Mech. Age. Dev., 34: 191, 1986.

23. Mishell, B. B. and Shiigi, S. M., Selected Methods in Cellular Immunology, W.H. Freeman, San Francisco, 1980.

24. Narayanareddy, K. and Murthy, P.B.K., Evidence for cytotoxic effect of vitamin E on human lymphocytes in vitro, Nutr. Rep. Inter. 26: 901, 1982.

25. Andersson, J., Moller, G. and Sjoberg, O., Selective induction of DNA synthesis in T and B lymphocytes, Cell. Immunol. 4: 381, 1972.

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26. Andersson, J. and Melchers, F., Lymphocyte stimulation by concanavalin A, in: Concanavalin A as a Tool, eds. H. Bittiger and H.P. Schnebli, p. 505, J. Wiley and Sons, London, 1976.

27. Spieker-Polet, H., Cruise, S.A. and Polet, H., The effect of serum albumin and the effect of cell concentration on the in vitro growth of mouse and rat lymphocytes, Cell. Immunol. 44:144, 1979.

28. Koren, H.S. and Hodes, R.J., Accessory cell functions of mouse tumour cells in the generation of cytotoxic lymphocytes in vitro: replacement of adherent phagocytic cells by tumour cells or 2-mercaptoethanol, Eur. J. Immunol. 7: 394, 1977.

29. Farrar, J.J., Mizel, S.B., Fuller-Farrar, J., Farrar, W.L. and Hilfiker, M.L. Macrophage-independent activation of helper T cells, J. Immunol. 125: 793, 1980.

30. Yasunaga, T., Kato, H., Ohgaki, K., Inamoto, T. and Hikasa, Y., Effect of vitamin E as an immunopotentiation agent for mice at optimal dosage and its toxicity at high dosage, J. Nutr. 112: 1075, 1982.

31. Bellas, R. and Corwin, L. M., Factors affecting enhancement of Con A-mitogenesis by vitamin E, Immunobiol. 163: 172, 1982.

32. Takenaga, K., Honama, Y. and Hozumi, M., Inhibition of differentiation of mouse myeloid leukemia cells by phenolic antioxidants and α-tocopherol, Gann 72: 104, 1981.

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TITLE:(U) MODIFICATION OF MITROGEN-INDUCED PROLIFERATION OF MURINE SPLENIC LYMPHOCYTES BY IN VITRO TOCOPHEROL

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ABSTRACT: (U) The effect of alpha-tocopherol on in vitro proliferation of murine splenic lymphocyte cultrues supplemented with various concentrations of teh vitamin has been measured at sub-optimal, optimal and supra-optimal levels of the T-cell mitogen Concanavalin A (Con A). In the concentration range (1-25 mug/m1), tocopherol enhanced proliferation when administered up to 24 hours after exposure to sub-optimal and optimal concentrations of Con A: however, at supra-optimal levels of the mitogen, it appeared to inhibit proliferation. In the concentration range 50-100 mug/m1, tocopherol supplementation only enhanced proliferation in response to sub-optimal concentration of Con A. The spontaneous proliferation of lymphocytes in the absence of mitogens was increased by tocopherol supplementation at all concentrations tested. In contrast, there appeared to be only slight stimulation of B-cell proliferation in response to optimal concentration of bacterial lipopolysaccharide (LPS) by lower levels of vitamin E. Tocopherol supplementation of cultures over a broad range of concentrations (0.5 - 100 mug/m1) had no significant effect on cell viability before onset of proliferation at 18 hours after exposure to Con A, nor was there evidence of earlier onset of DNA synthesis in response to mitogen in the presence of 5 mug/m1 of the vitamin. TRUNCATED

DESCRIPTORS: (U) Lymphocytes;Immunology;Tocopherol;Antigens;Vitamins;Cells (biology);Cultures (biology)
IDENTIFIERS: (U) Interleukins;Mitogens;Immune response

REPORT DATE: 01 Jan 1991 **NUMBER OF PAGES :** 20

Reprints: Immunopharamacology and Immunotoxicology, vol 13, no 4, 1991, p 531-550

DRDKIM N°: 96-02597 **CANDIS N°:** 157124 **Format(s):**Document Image stored on Optical Disk;Hardcopy